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Correlations of Ultrastructure and Pigmentation Suggest How Genes Control Development of Wing Scales of *Heliconius* Butterflies

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Introduction

In the last two decades there have been extensive genetic studies of the mimetic wing patterns of *Heliconius* butterflies (Turner, 1981; Sheppard *et al.*, 1985), but these analyses have not probed the precise nature of color patterns at the ultrastructural level. Thus, while many ecological and evolutionary phenomena have been elucidated, the nature of the genes involved and the mode of their action in the course of wing pattern development remains obscure.

One another front there is renewed interest in general models of pattern formation in butterfly wings (Nijhout, 1986), but studies of pattern development have relied upon comparisons among related species or upon experimental manipulations of wing development within species. Remarkably, there has been a lack of genetical analysis applied to the problem of butterfly wing pattern development on the one hand, and little attention given to the chemical and ultrastructural basis of "color pattern" on the other, although excellent studies have been carried out on chemistry (e.g., Umebachi and Yoshida, 1970; Descimon, 1975) and ultrastructure (e.g., Ghiradella, 1974; 1985) separately.

In an attempt to refine genetic hypotheses which explain the variation of wing pattern observed in crosses involving different races and species of *Heliconius* (Sheppard *et al.* 1985; Gilbert, in prep.), it appeared

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appropriate to investigate the chemical and morphological basis for color and pattern. This paper summarizes our initial investigations of wing scale structure and chemistry on four species of *Heliconius* which have been subjects of genetic studies in the senior author's laboratory.

The findings presented for *Heliconius* below provide the first clear evidence from lepidopteran wings that genetic control of pigmentation patterns simultaneously involves patterns of differentiation in scale ultrastructure, a result anticipated in general terms by Descimon (1965). Thus, beyond elucidating the connections between genes and wing patterns in butterflies, the results suggest that *Heliconius* wings may provide a useful system for addressing general questions about the genetics of pattern development.

Material Examined

All butterflies for ultrastructural and chemical studies were reared in greenhouses at Patterson Laboratory, The University of Texas at Austin. *Heliconius* species examined included *Heliconius cydno galanthus* (stock origin, La Selva, Costa Rica), *Heliconius pachinus* (stock origin, Osa Peninsula, Costa Rica), *Heliconius melpomene rosina* (stock origin, Osa Peninsula, Costa Rica), and *Heliconius ismenius clarescens* (stock origin, Osa Peninsula, Costa Rica). Hybrid "bar-shadow" regions (explained below) were from F1 hybrids of the *H. cydno* and *H. pachinus* stocks above. In addition, forewing red/brown scales were examined in *H. cydno* — *H. melpomene* crosses. Illustrations of these species may be found in DeVries (1987) and of their hybrids in Gilbert (1984).

Because a wide variety of methods are used in this study, necessary details of techniques will be provided below.

SCALE MORPHOLOGY

Scales were examined by standard methods of scanning electron microscopy. Dry wing fragments with uniform scale color were coated with 25Å of gold-palladium in a Hummer V sputter coater and examined at 600 and 10,000X using an ISI Super IIIA. Scale cross sections were created by cutting wing fragment with a razor blade and searching that area for appropriately cut scales.

Descriptive terminology used below follows the system developed by Downey and Allyn (1975). It should also be stressed that while we are confident in distinguishing the following major scale types, interpretation of many morphological details is tentative.

Type I scales. Yellow/white (Fig. 1 A, B, C, D; Fig. 5B; Fig. 6A)

In *Heliconius*, yellow and white scales appear to represent the same morphological type. Average spacing of scute peaks (=lamellae of Ghiradella, 1985) along the ridge is approximately the same as the inter-ridge distance. Obverse membrane obscures the scale internal

structure. Many variable sized windows may occur in the membrane, especially in the central region of the scale. Transverse flutes (=microribs of Ghiradella, 1985) run over the membrane surface between, and perpendicular to, longitudinal ridges. Such flutes are evenly spaced and occur at a density of 8-10 per inter-scute interval.

White and yellow scales appear to lack crossribs, and scale cross sections show trabeculae primarily below longitudinal ridges.

The spacing of ridges in Type I scales is narrower on the dorsal wing surface, so that dorsal wing scales often have 1.5 to 2 times the number of ridges per scale width as do ventral wing scales (compare Fig. 1B vs. 1A). This ultrastructural difference may help account for the sheen and richer colors of the dorsal versus the ventral wing surfaces.

Type II scales. Black (Fig. 2A, B, C, D; Fig. 5A, C; Fig. 6B)

The melanic scales of *Heliconius* possess longitudinal ridges connected by ladder-like crossribs, most of which are supported by trabeculae. Crossribs appear more narrow than ridges and are arranged in poorly aligned rows. There is usually no obverse membrane in melanic scales and flutes are visible only on the vertical walls of longitudinal ridges.

As in Type I scales, ridges are spaced more widely on ventral wing scales than on dorsal scales. In both *H. cydno galanthus* and *H. pachinus*, dorsal scales noted as "dull" proved to have more widely spaced ridges than those noted to be "shiny" (e.g., Fig. 2C vs. 2B).

Type II′ Hybrid "bar-shadow" scales. Black (Fig. 3A, B, C)

The "bar shadow" is a region of altered reflectance on the melanic region of a hybrid *Heliconius* ventral hindwing. This region corresponds to the location of yellow scales in one parent race of a cross, the other parent of which possess a totally melanic hindwing. The bar shadow is used to diagnose hybrid genotypes in ecological genetic studies (Mallet, 1986).

These scales are identical to Type II scales except that roughly 5% of spaces between crossribs are covered by obverse membrane (Fig. 3). In some cases the membrane is intact over the inter-rib space, but in most cases these scales resemble partly dissolved tissue draped over chicken wire. This subtle change is visible to the naked eye, but not under light microscope. This scale type might be viewed as a small step toward a morphological hybrid of Type I and Type II scales. However the membrane, where present, lacks supportive flutes and the scales are otherwise identical to Type II scales.

Type III scales. Reds and browns (Fig. 4A, B, C, D; Fig. 5D; Fig. 6C)

"Red and brown" scales in *Heliconius* include orange, orange-brown, brown, pink, and red scales. These share a basic morphology, Type III. Crossribs often appear to be wider than those of Type II scales, and one

or two strengthening flutes pass over each crossrib, connecting adjacent ridges. In addition, obverse membrane appears to be retained over each crossrib and immediately adjacent to longitudinal ridge. This may account for the thicker appearance of ridges and ribs in Type III scales, as well as the angular appearance of crossribs.

Cross sections of Type III scales do not reveal trabeculae supporting presumptive crossribs, but they can be seen supporting longitudinal ridges, and may simply occur less frequently than in Type II scales. Without further detailed work, it is not possible to exclude the possibility that strips of membrane supported by flutes function as pseudo-crossribs rather than overlay them as suggested above. Close examination of Type III scales suggests their closer relationship to Type I than to Type II scales. For example, the inter-ridge space on the left extreme of the brown scale in Fig. 4A is virtually identical to the obverse membrane of a dorsal Type I scale (e.g., Fig. 1B). These characteristics gradually change to typical Type III features toward the center of the scale. Like Types I and II, Type III scales typically possess more narrow spacing of the longitudinal ridges on the dorsal wing surface.

Scale Chemistry

White Scales: No pigment

White scales of *Heliconius oydno* possess a highly reflective quality or sheen quite unlike the flat white of *Pieris*. Under light microscope at low power, these scales are brighter where two or more overlap. These observations suggested a structural rather than chemical basis for the white color.

To test this possibility, scales were immersed in a solution whose refractive index is near that of chitin (1.55). When single scales were observed in such a solution (xylene or Permount) against a black background, they became essentially transparent. Scales in xylene regain their white luster when the liquid evaporates. Comparisons with yellow and black scales indicate that luster, but not color, disappears in these liquids. Uric acid tests were negative on chromatographs of Type I scale areas. White scales in *Heliconius* are therefore due to structural features of the scale rather than pigments.

Yellow Scales: 3-hydroxykynurenine

A small circle of the yellow part of the wing was cut out with a cork borer, and positioned carefully at the aperture of the Cary Recording Spectrophotometer. Measurement of the UV spectrum revealed peaks at 282nm and 405nm (a rather broad peak). These are essentially identical to the peaks produced by 3-hydroxykynurenine in 0.1N NaOH (pH 14) (285 and 395nm).

Extraction of the pigment using water or dimethyl sulfoxide and

rerunning the spectrum of this extract in 0.1N NaOH or 0.1N HCl gave the following values:

λ *max 0.1N NaOH*	λ *max 0.1N HCl*
280(285)	252(252)
395(395)	312 shoulder(312)

3-hydroxykynurenine peaks (in parentheses)

These data indicate that the yellow pigment in *Heliconius* spp. is the alkaline form of 3-hydroxykynurenine. This pigment is previously described from *Heliconius* (Brown, 1967).

An interesting question concerns the maintenance of 3-hydroxykynurenine in its alkaline form in the wing scales of *Heliconius*. Chromatographic studies were carried out to elucidate this phenomenon. Fragments of yellow wing areas of *H. pachinus* were ultrasonicated, then agitated in 80% methanol. This extract was spotted on Whatman No. 1 filter paper and subjected to one dimensional chromatography using BAW, n-butanol/acetic acid/water (4:1:1) as solvent. A ninhydrin test (1g of ninhydrin dissolved in 50ml acetone; chromatograph was immersed in this solution, allowed to dry, and heated at 110°F until color developed) revealed that an amino acid or peptide was located in the identical spot with the alkaline form of 3-hydroxykynurenine (revealed by UV light).

A comparison with white scales using the same procedure showed the identical ninhydrin sensitive spot, but no yellow pigment. Two-dimensional chromatographic studies of all basic amino acids using the same solvent, BAW, did not duplicate the spot derived from *Heliconius* wing extract. It is therefore likely that a peptide or small polypeptide is responsible for keeping 3-hydroxykynurenine in the alkaline state. The precise location of this complex within the scale is not yet determined.

Black Scales: melanin

Chromatographic evidence verified that black scales contain melanin but tryptophan is also present in extracts of black scales. Melanic scales embedded in paraffin, sectioned, and examined with light microscope revealed that pigment is found in the walls of ridges and in crossribs.

Brown and Red Scales: xanthommatins

Wings were extracted with dimethyl sulfoxide (DMSO) or with 2% HCl in methanol. The spectrum of the extracts and of standard compounds are given in Table 1. There are two problems of interpretation with these data. First, the spectra of xanthommatin and dihydroxanthommatin are notoriously difficult to reproduce because of rapid decomposition and slight changes in state of reduction (see Linzen, 1974). Second, it was not possible to make comparisons of extracted

Table 1. Extraction methods and absorbance values (nm) of chemical standards and wing pigments of *Heliconius* (*Values from Denys, 1982)

PIGMENTS	EXTRACTION	ABSORBANCE MAXIMA (nm) CONDITIONS						
		DMSO	pH7-7.5	Acid Methanol	5N HCl	2% Digitoxin pH6.5	2% Digitoxin pH10.4	
Brown from H. ismenius	DMSO	440-450,365						1
	DMSO acidified to 5N HCl				450,360			2
	acid methanol			450, no distinct peak at 360				3
Red from H. melpomene	DMSO	490,365						4
	DMSO extract + acetone/ether; resulting ppte. in H2O pH7.0		465,368					5
	acid methanol			448, no distinct peak at 360				6
Red from H. pachinus	acid methanol			458,360-380				7
	acid methanol, oxidised with NaNO2			448				8
Brown from pure sample xanthommatin	dissolved as indicated		440		475-480, 370-375	*450	*478	9
Red from pure sample dihydro-xanthommatin	dissolved as indicated		505-510	475-360	500(shoulder), 390			10
	Reduced with NaBH4			490,370				11
		1	2	3	4	5	6	

pigments from *Heliconius* wings with standard samples of xanthommatins under absolutely identical or controlled conditions. However, taken as a whole, the data indicate first that the major brown or red pigments of *Heliconius* butterflies are xanthommatin and dihydroxanthommatin and second, that variations in color from bright red to brown are due to variations in the state of oxidation of dihydroxanthommatin (or the state of reduction of xanthommatin).

First note the correspondence in the spectral maximum between the brown pigment of *H. ismenius* and that of xanthommatin (Table 1, row 1 and 2 versus row 9). Further note the DMSO extract of *H. melpomene* red pigment (Table 1, row 5, col. 1), the spectrum of which peaks near that of dihydroxanthommatin under reduced conditions (Table 1, row 11, col. 3). Obviously these extracts of red dihydroxanthommatin are in various stages of oxidation to xanthommatin.

Given the in vitro instability of the reduced red form of xanthommatins, the observed stability of various shades of orange and red on the wings of various races of *H. melpomene* and *H. erato* presents an

interesting mystery. However, the likelihood that 3-hydroxykynurenine is maintained in an alkaline state by a gene product suggests that such associations may allow races to "select" localized pH conditions within Type III scales by modification of a peptide or protein associated with xanthommatin pigment, and thereby affect subtle variation in the actual color displayed on the wing.

Evidence from *H. cydon X H. melpomene* crosses indicates genetic control of separate factors maintaining the reduced form of xanthommatin (red) in *H. melpomene*. Thus, in all F1 hybrids of *H. pachinus* or *H. cydno* with red forewing banded *H. melpomene* races (illustrated in Gilbert, 1984), brown scales appear on the ventral side of the dorsal forewing red band, and with appropriate crosses, one can convert the dorsal red forewing band of hybrids to brown (Gilbert, unpublished data).

Discussion

Different hypotheses can be proposed for how genes determine final color patterns in *Heliconius*. At one extreme, scale morphology and pigmentation would be separately determined by independently regulated genes such that any combination of structure and color could occur. This possibility is not the case in *Heliconius* because of melanic scales (including bar-shadow scales), which are consistently found to have a particular subset of ultrastructures, scales with Type I ultrastructure which consistently lack melanin or xanthommatin, and Type III scales are never white, yellow, or melanic.

At the other extreme, genes which determine pigment production in a developing scale might pleiotropically determine its ultrastructure. This would be the case if the product of a single gene directly or indirectly regulates both morphological events and the pigment pathway within a scale. In *Heliconius*, this possibility appears to hold true at the level of major pigmentation differences (eg. xanthommatin vs. melanin). However, some pigment variation such as brown vs. red in Type III scales, or white vs. yellow in Type I scales, represents minor pigment variation within the major categories. We hypothesize that such minor variation in Type III scales is based on variation in genes coding for those peptides which act to stabilize pigments at particular oxidation states in the scales.

Any useful model for scale development in *Heliconius* should explain the observed correlations of structure and pigmentation (summarized in Table 2) in genetic and chemical terms. It should also be in general accord with current knowledge of scale development, pigment chemistry, and genetics. Fortunately, development of scale pigmentation has been carefully studied in another nymphalid genus, the pigments involved are relatively well-studied in other systems, and extensive classical genetics is available for *Heliconius*.

With respect to scale development, Nijhout's (1980) observations and experiments provide a useful model for the development of different colored melanic scales in *Precis* (Nijhout, 1980, p.287).

1. Enzymes for pigment synthesis are insoluble but active within cuticle of the scale.
2. Substrates for pigment synthesis circulate in the hemolymph and are produced in sequence.
3. Substrates can gain access to scales at all times.
4. Scales in each presumptive color region possess only a single enzyme and are capable of utilizing only a single substrate.

Nijhout (1980) also observed that longitudinal ridges formed before melanin deposition, indicating that the pigment per se only stiffens the scale, but does not direct its morphogenesis. We therefore assume that in *Heliconius*, any pleiotrophic effects of genes involved in pigment pathways on scale structural distinctiveness is not via the pigment, its precursors, or substrates. Rather it seems most likely that the product of a "scale selector" gene acts as a turn-on switch for other genes involved in scale ultrastructure on the one hand, and genes for pigment pathway enzymes on the other.

Our interpretations of the chemistry of yellow, red, and brown variation in *Heliconius* benefit from the extensive genetic and biochemical work on *Drosophila* eye color variation which is based on the same ommochrome pathway (Summers *et al.*, 1982). Xanthommatin pigments derive from tryptophan via intermediates such as kynurenine (Linzen, 1974), but two lines of evidence suggest that the substrate for xanthommatin production is 3-hydroxykynurenine. First, in *Drosophila* eyes, normal xanthommatin production depends on external kynurenine and/or 3-hydroxykynurenine supplied via the hemolymph (Summers et al., 1982). Second, Linzen (1970) reviews evidence that a) in holometabolous insects, including Lepidoptera, tryptophan accumulation in hemolymph and other tissues is transitory and b) 3-hydroxykynurenine is the metabolite most likely to persist at elevated levels. Thus, it is reasonable to assume that the substrate for xanthommatin in *Heliconius* Type III scales is 3-hydroxykynurenine.

Similarly, although melanins arise ultimately from oxidation of tyrosine, the substrates for melanin production are likely to be dopa or dopamine if *Heliconius* follows the usual pattern for insects (Wigglesworth, 1972) and for *Precis* wing scales (Nijhout, 1980).

Association of xanthommatin and other ommochromes with specific proteins in silkworm blood (see Linzen, 1974 for review) make our suggestions about mechanisms of color fine-adjustment and stability a credible working hypothesis. On the other hand, reports of ommochrome-binding protein in cecropia moth eyes (Ajami and Riddiford, 1971) have not been verified in parallel studies on *Drosophila* (Wiley and Forrest, 1979), nor have the subtle variations in *Drosophila* eye color been adequately explained.

Additional parts of the *Heliconius* scale puzzle are provided by genetic evidence. Certain genes for xanthommatin scales (Type III) are dominant (Sheppard *et al.*, 1985) or epistatic (in single dose) to those for melanic scales (Type II) (Gilbert, in prep.) in *H. melpomene*. Other dominant or epistatic genes, active in the same regions of the wing, may replace yellow scales (Type I) with melanic or xanthommatin containing scales (see Sheppard *et al.*, 1985). We suggest these observations are due to our hypothesized scale selector genes, the interaction of which generally produces an unambiguous scale type in the following order of dominance or epistasis: III > II > I. Bar shadow scales on the ventral hindwing may represent an exception to this rule if they indeed possess intermediate features.

Genetic variation for pigmentation within scale types appears to have no common theme. In Type I scales, white is dominant to yellow (see F1 of *H. cydno* X *H. pachinus*, (Gilbert, 1984). This is counter to what we would expect if the heterozygote simply possesses one half the amount of yellow pigment. We hypothesize a gene involved with transport of 3-hydroxykynurenine into the developing Type I scale. The bar shadow variant of Type II scales (II') probably reflects dosage of Type II selector gene, M, (but only expresses on one wing surface!). Color variation of xanthommatin pigments may be due to a structural gene for the binding peptide as previously discussed.

At this stage of knowledge, many alternative models of *Heliconius* scale development might be equally difficult to reject. With this caveat, we present a model which is consistent with the observed relationships of scale structure and color (Table 2) and which assumes as valid, the foregoing points about scale development, pigment chemistry, and genetics. Finally, for simplicity, we develop the model as a series of binary choices which depend upon the state of scale selector genes in cells which give rise to the scales. The following model should be considered a tentative scheme rather than a well-substantiated theory.

During the course of development, cells would be fated to give rise to a particular scale type at a particular wing location by the combination of selector genes which are switched on or off. The threshold conditions for such switching might allow trichogen cells of the same genotype to end up as different scales types depending upon the strength of morphogen signals at that location (see Nijhout, 1986).

A fundamental decision in scale development seems to be between Type I versus Type II or III, because Type I scales do not require pigment to stiffen, and are apparently not manufacturing complex pigments from simple substrates. For simplicity, we consider this scale type the null state, that scale type which develops if no other selector genes are switched on.

Next, if Type II selector switch gene M is turned on, cells are fated to develop Type II or Type III scales. Given that only M is on, and given appropriate positional information, the M + signal would turn on

Table 2. A summary of the relationship between scale morphological types and scale pigmentation observed in the four *Heliconius* species and two interspecific crosses of this study. Type II′ refers to the bar shadow scale type, yellow, brown, and red refer to 3-hydroxykynurenine, xanthommatin, and dihydroxanthommatin respectively (see text).

SCALE TYPE	PIGMENTATION: none	yellow	brown	red	melanic
I	X	X			
II					X
II'					X
III			X	X	

morphological programs and melanin pathway enzymes. However, the scale would not melanize and stiffen until dopa or dopamine circulate in the hemolymph.

In keeping with a binary decision model of genetic determination, we suggest that the selector gene for Type III scales, X, can only be expressed in M + cells, and that its signal initiates Type III morphology and turns on genes for xanthommatin pathway enzymes. Genetic evidence summarized above indicated that M++X+ cells give rise to Type III, xanthommatin containing scales.

Thus, it appears that the xanthommatin pathway inhibits in melanin pathway by a method similar to its inhibition by another oxidative pathway, xanthopterine synthesis (Wigglesworth, 1972). Since in *Heliconius* pupae, homozygous and heterozygous forewing Type III patches develop xanthommatin well ahead of the melanization of Type II areas (Gilbert, unpublished observation), it may be that the pigment itself inhibits the oxidation of substrates of the melanin pathway as is the case with xanthopterine and melanin. In explaining the epistasis of X over M in determining scale morphology, it may be less complicated to assume that morphology is a direct result of pigment-scale interaction. However, as one reviewer pointed out, in the absence of further information, independent determination is a better null hypothesis. In our model therefore, the X+ signal overrides M++ to redirect morphogenesis, and acts separately on genes involved with morphology and pigmentation as M is hypothesized to do.

This scheme of developmental genetic control is summarized by Figure 7. This diagram also shows the final genetically controlled decisions which occur after scale type is established which we have discussed above. For each branch, the gene dosages necessary for each state of a scale is indicated by plus (one gene dose) or zero (null).

Summary

Scanning electron microscopy reveals three morphological categories of wing scales in *Heliconius* butterflies. Type I, white or yellow scales, possess an obverse membrane between longitudinal ridges and lack conspicuous crossribs. Type II, melanic scales, have ladder-like, regular crossribs supported by trabeculae. Type III, red or brown scales, are characterized by crossribs which feature flutes and a thicker, more angular appearance. In hybrids, whose parents possess Type I and Type II scales on the hind wing bar respectively, the "bar shadow" scales which replace the yellow bar appear to be a slightly modified version of Type II scales.

Spectroscopic analyses reveal that yellow, red, and brown pigments are tryptophan derived 3-hydroxykynurenine, dihydroxanthommatin, and xanthommatin, respectively. White is a structural color expressed when yellow pigment is not present, while red and brown are different oxidation states of xanthommatin. Chromatographic evidence suggests the possibility that unstable forms of pigments in this pathway are maintained by association with peptides in the scale. Thus, although substantial color variation occurs within scale morphological types, it is chemically trivial. These observations are supplemented by evidence from the literature to develop an hypothesis for the relationship between genes, scale pigmentation, and scale structure (Figure 7).

Because of the variety of scale morphology and pigment chemistry within the Lepidoptera, it is not possible to assess the degree to which this scheme for *Heliconius* wing color pattern constitutes a model for other groups. However, it will be surprising if the *Heliconius* system described here turns out to be other than a variation on a theme common to all butterflies and moths. Indeed, a similar correlation of color and structure has been described for zygaenid moths (Burgeff and Schneider, 1979).

More generally, *Heliconius* wings may contribute to some of the unsolved problems of the genetics and development of tissue specific ommochrome pigmentation. This is because one can work with scale specific regulation of the pathway on the wings within species having distinctively patterned genotypic varieties or races, rather than rely on constitutive mutants.

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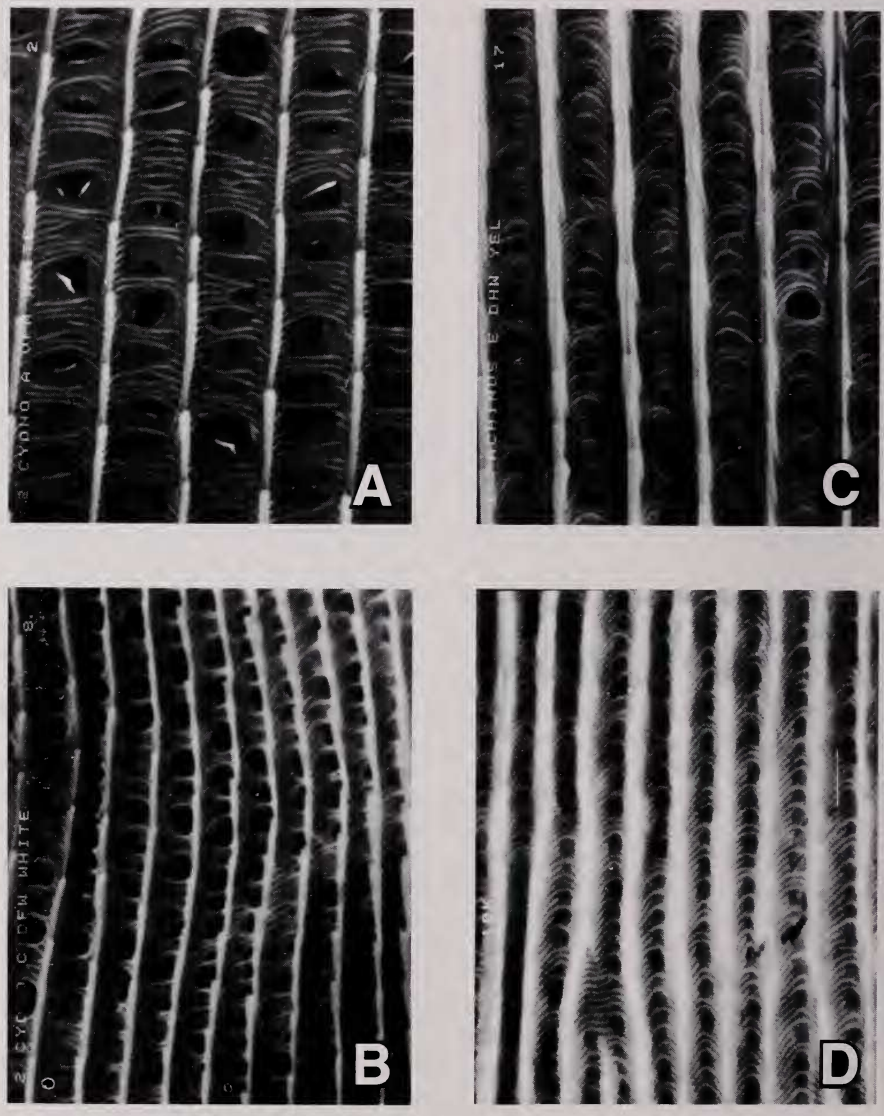

Fig. 1. Type I, white or yellow scales. All cover scales viewed perpendicular to surface at 10k. A. White scale, ventral forewing, *H. cydno galanthus*. B. White scale, dorsal forewing, *H. cydno galanthus*. C. Yellow scale, dorsal hindwing, *H. pachinus*. D. Yellow scale, dorsal forewing, *H. pachinus*. Note on bottom left of D, where ridge spacing increases, obverse membrane of dorsal scale resembles that of a ventral scale.

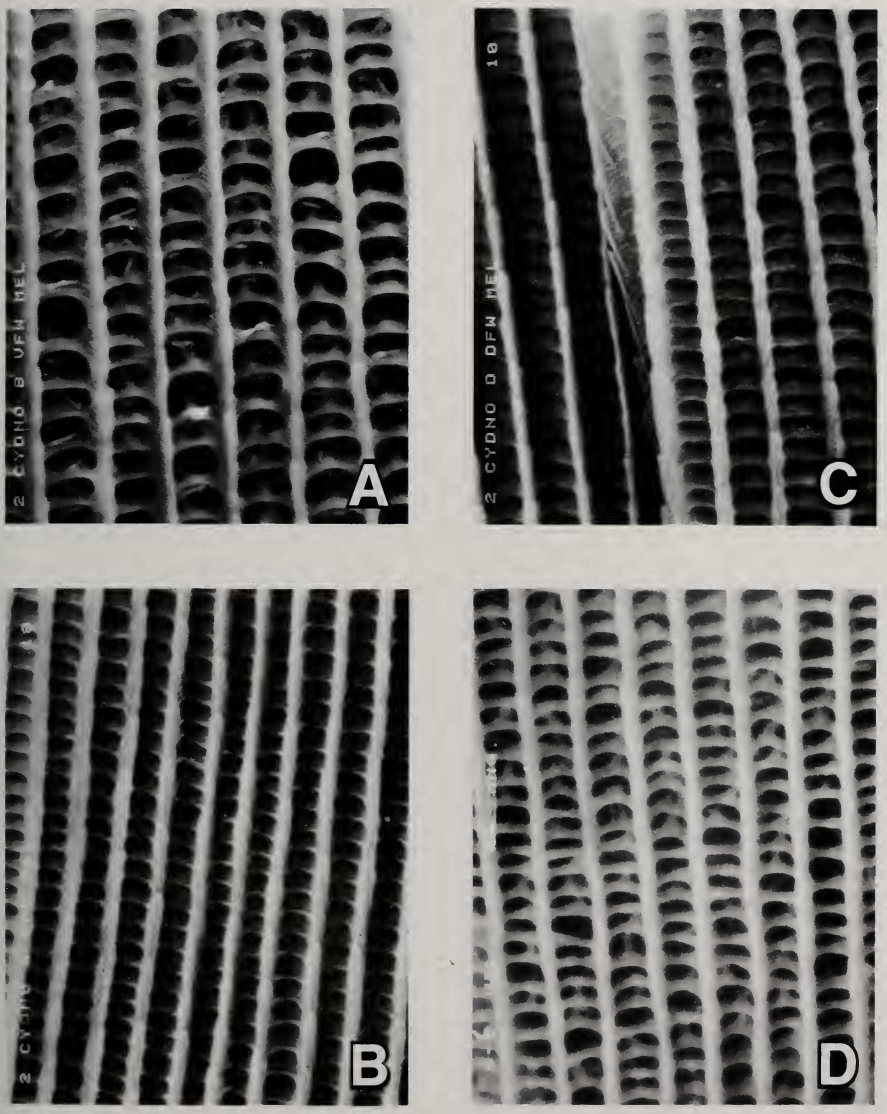

Fig. 2. Type II, melanic scales. All viewed approximately perpendicular (±10°). A. Ventral forewing, *H. cydno galanthus*. B. Dorsal hindwing (shiny scale), *H. cydno galanthus*. C. Dorsal forewing (dull area), *H. cydno galanthus*. D. Dorsal forewing, *H. ismenius*.

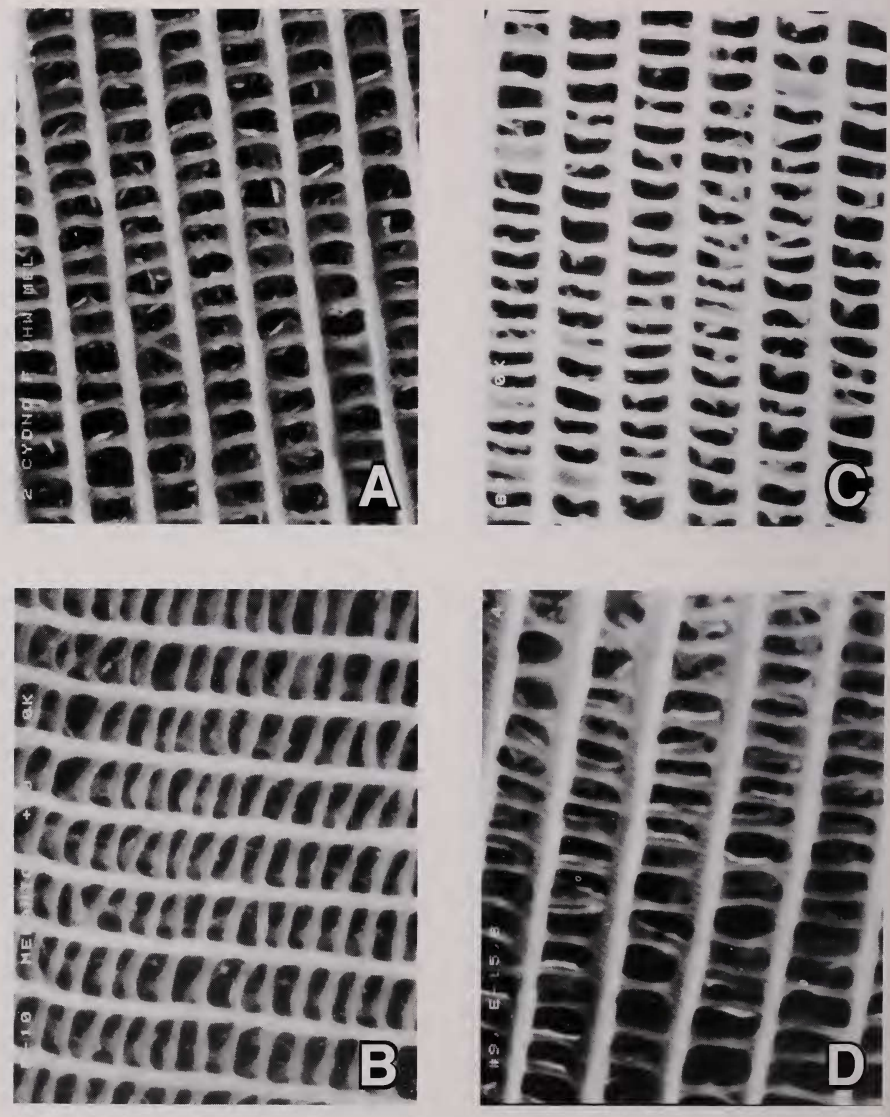

Fig. 3. Type II, "shadow" scales. These ventral hindwing scales lie in zones of altered reflectance and are diagnostic of hybrids between forms with yellow hindwing bars X forms with all black hindwings. A. Shadow region of a *H. cydno galanthus* X *H. pachinus*, F1 hindwing. B. Non-shadow region of a *H. cydno galanthus* X *H. pachinus*, F1 hindwing. C. Shadow region of *H. cydno galanthus* X *H. pachinus*, F1 hindwing, D. Shadow region of *H. cydno galanthus* X *H. pachinus*, backcross hindwing.

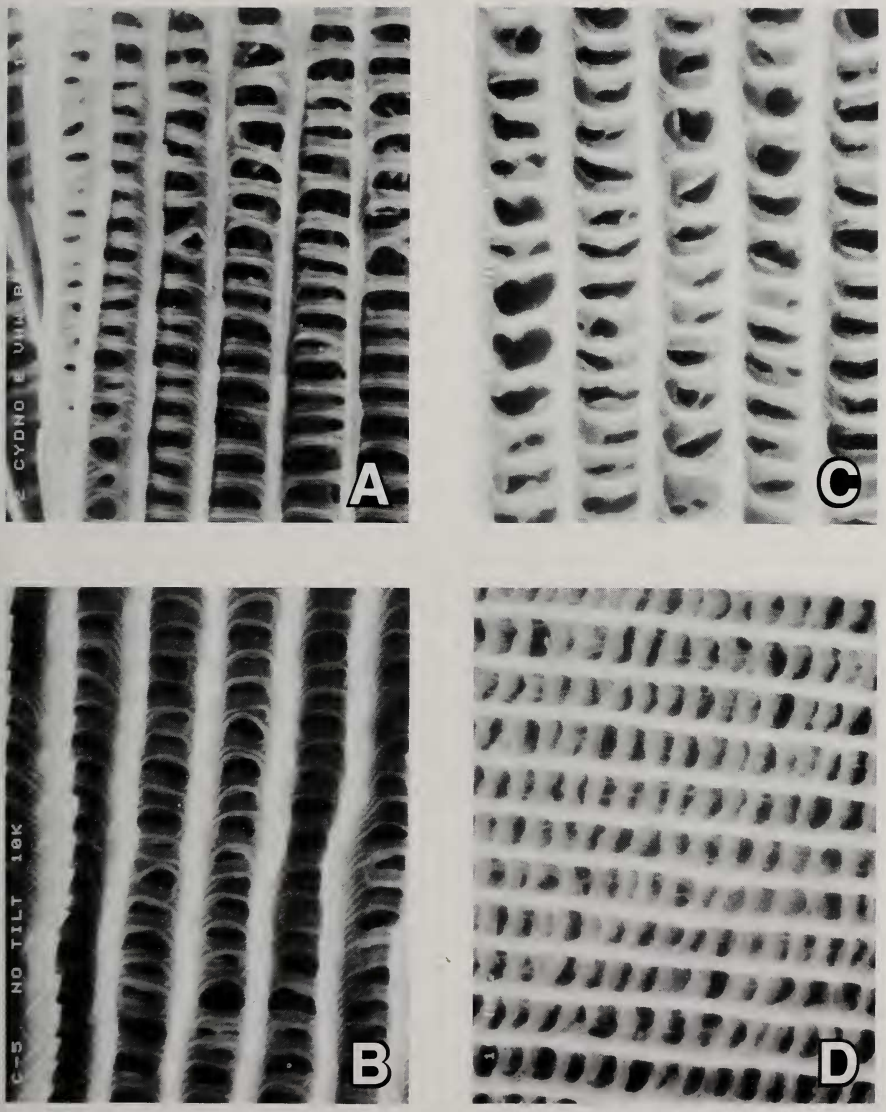

Fig. 4. Type III, red or brown scales. A. Ventral hindwing, *H. cydno galanthus* (brown). B. Ventral hindwing, *H. pachinus* (basal red spot). C. Ventral forewing, hybrid *H. cydno* with *H. melpomene* forewing band (brown). D. Dorsal forewing, hybrid *H. cydno* with *H. melpomene* forewing band (brown).

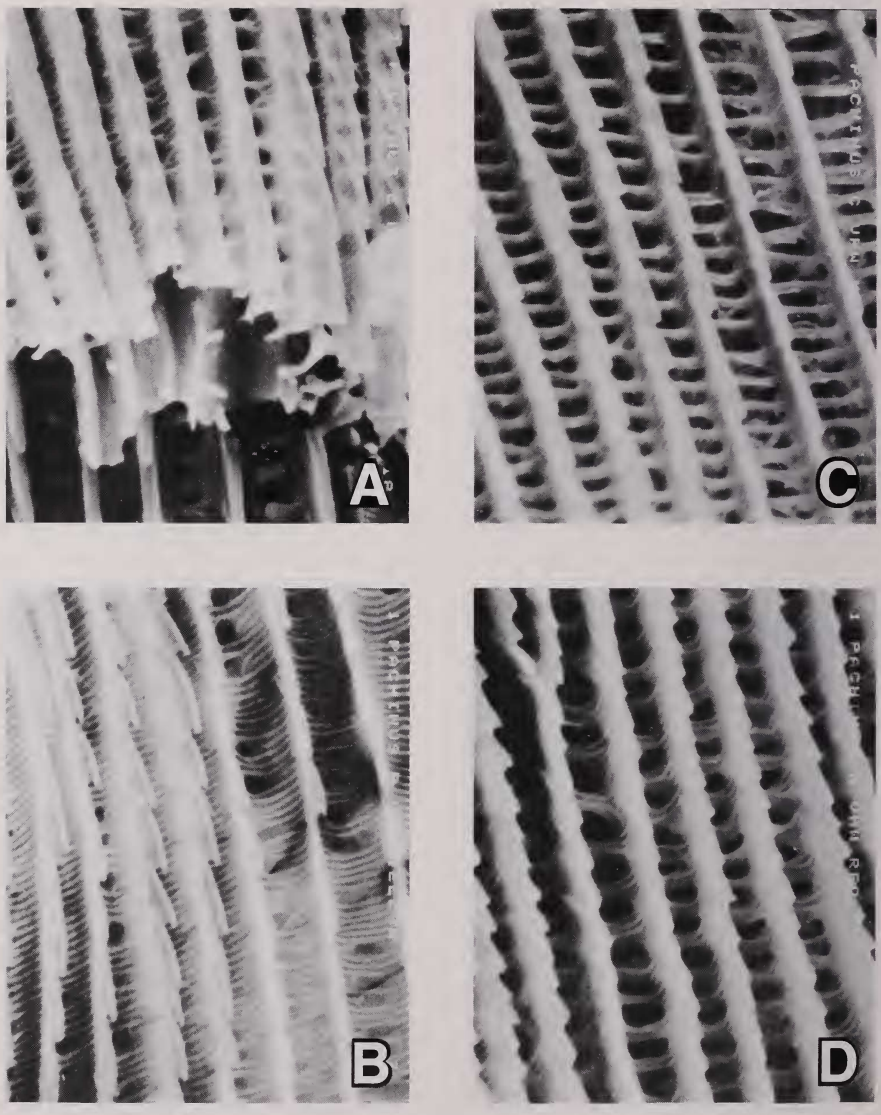

Fig. 5. Angled views of various scale types (all of *H. pachinus*) showing scutes, all at 10k. A. Dorsal hindwing, melanic, *H. pachinus*. B. Ventral hindwing, yellow, *H. pachinus*. C. Ventral hindwing, melanic, *H. pachinus* D. Ventral hindwing, basal red scale, *H. pachinus*.

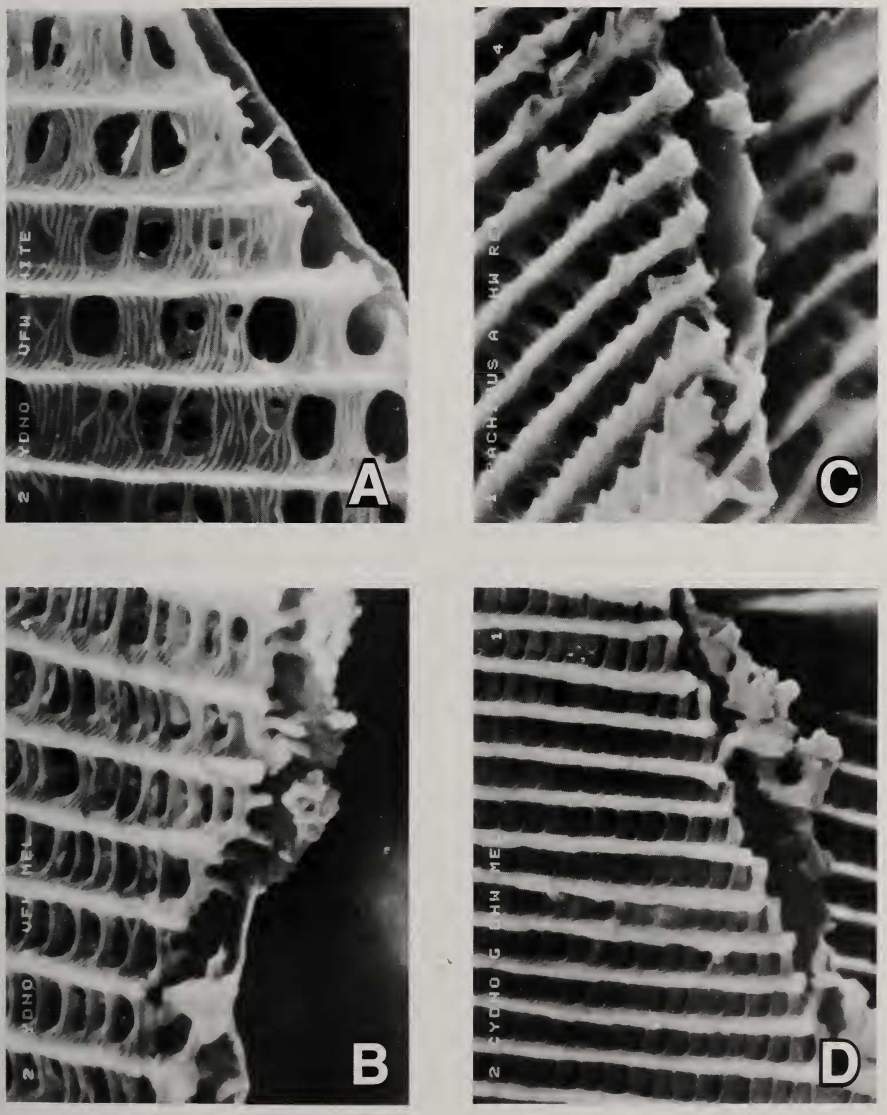

Fig. 6. Cross sections of various scale types, all at 10k. A. Ventral forewing, white (Type I), *H. cydno*. B. Ventral forewing, melanic (Type II), *H. cydno*. C. Basal red spot, ventral hindwing, *H. pachinus*. Dorsal hindwing, melanic, *H. cydno*.

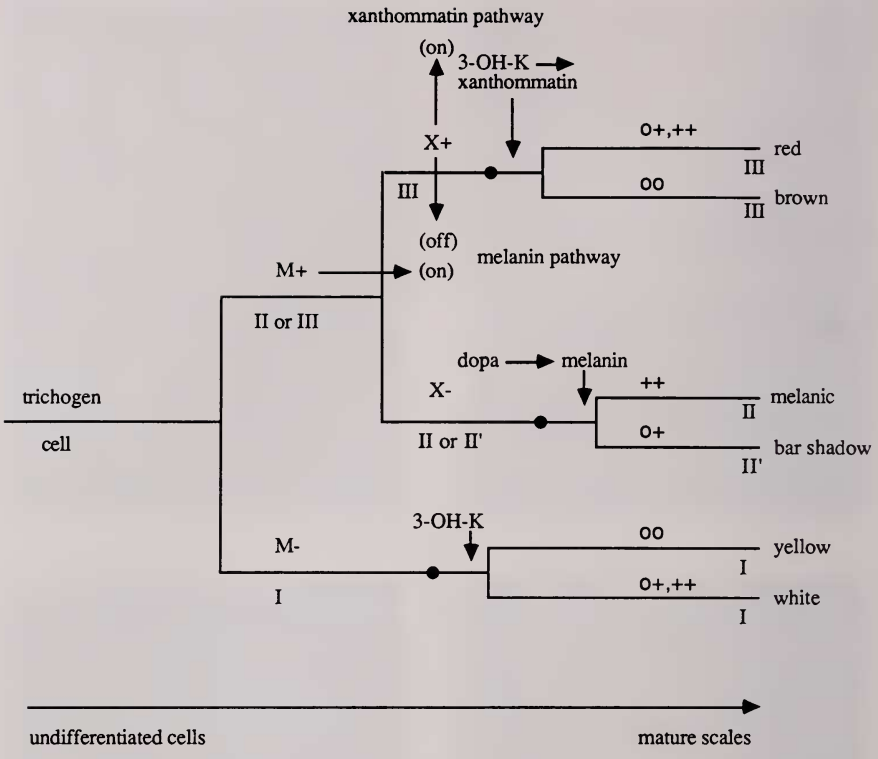

Fig. 7. Hypothetical scheme for genetic control of *Heliconius* wing scale development based on morphological, chemical, and genetic information discussed in text. Solid circle represents time that morphological characteristics of mature scale begin to be established. M and X are selector genes regulating morphological decisions and pigment pathways as shown. Effect of genes which act within major scale categories are indicated on the final branches of the diagrams in terms of doses (indicated by "+"). Type I scales vary in terms of a gene which affects transport of 3-hydroxykynurenine (3-OH-K) to the developing scale, one dose (o+) gives a white scale. Type II' scales probably represent scales heterozygous for M (o+). Type III scales vary according to a structural gene for a pigment binding peptide. One dose (o+) stabilizes xanthommatin in its reduced state. (See text)